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B. T. GALLOWAY, *Chief of Bureau.*

NEW METHODS OF PLANT BREEDING.

BY

GEORGE W. OLIVER, Plant Propagator.

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FOREIGN SEED AND PLANT INTRODUCTION.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., September 25, 1909.

Sir: I have the honor to transmit herewith a manuscript entitled "New Methods of Plant Breeding," by Mr. George W. Oliver, Plant Propagator of this Bureau, and recommend that it be published as Bulletin No. 167 of the Bureau series.

Respectfully,

B. T. Galloway,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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NEW METHODS OF PLANT BREEDING.

INTRODUCTION.

A few years ago Dr. B. T. Galloway, Chief of the Bureau of Plant Industry, mapped out some plant-breeding work for the writer which involved crossing varieties of lettuce, alfalfa, and cowpea, and also certain species of Poa, Trifolium, Melilotus, etc. The work in the beginning presented many difficulties which have since been overcome, and it is now progressing satisfactorily.

The improvement of plants by cross-breeding varieties is gradually becoming an important factor in the culture of many of our field and garden crops. The majority of intentional crosses and hybrids made in the past represent work which has been easy of accomplishment. The more difficult subjects have been passed by or at most the efforts of breeders with the hitherto refractory genera have to a large extent been nullified by natural obstacles in the path of success.

The only difficult part of plant breeding lies in knowing just how to make the most of a cross or hybrid which has been secured. The literature on this subject which has appeared during the last few years is apt, unfortunately, to lead the beginner and even the practical breeder into a maze, and he may conclude that the subject is too complex for comprehension.

This should not cause discouragement, however, because the mission of the plant breeder is to produce varieties which are in some measure improvements over the old ones, and each advance made will tend toward that perfecting of plant life which will insure better products for man and the domestic animals.

The present contribution to the subject deals not with laws, but with methods used by the writer in accomplishing what have hitherto been considered impossible or difficult crosses, and they may prove helpful to others engaged in the same line of work.

Until recently it has been found impossible to cross many plants owing to the fragile nature of the sexual organs. This difficulty has been removed, and the process whereby it has been accomplished is here described for the first time.

There are numercus plants, also, both ornamental and economic, the flowers of which are so small that their stamens are very difficult

to remove in the process of emasculation, and this fact has undoubtedly contributed largely toward delaying the rapid improvement of many flowering plants, fruits, and vegetables by cross-breeding and hybridizing. Mendel, who did so much in his particular line, worked with two genera only: Pisum and Hieracium. The former is easy to manipulate, but all the species of Hieracium present difficulties in emasculation which by the ordinary methods are practically insurmountable. In fact, Mendel found them so great that he did not finish his work on this genus. Nevertheless, in the flowers of the hieraciums, and many other plants regarded as equally difficult, the pollen can be thoroughly removed, and with as great facility as in those of the rose or any other flower in which the reproductive organs are large and easily handled. This new process, which may be called "depollination," is the removal of pollen from the stigma before fecundation has taken place. It is applicable to flowers in which emasculation is impracticable because of the minuteness or delicacy of the floral organs. It is hoped that this newly found method of preventing fecundation by undesirable pollen will be instrumental in furthering the projects mapped out by the ever-increasing number of plant breeders.

The writer has worked on several genera of the Compositæ with complete success. The method here described can be used not only with the Compositæ, but also with all flowers having reproductive organs too small to be successfully manipulated by the ordinary methods of emasculation. The use of the method does not stop here. It can also be applied to the stigmas of larger flowers when there is any doubt whether pollen has recently gained access to the stigmas previous to artificial pollination.

ORIGIN OF THE NEW METHODS OF PLANT BREEDING.

During the spring of 1903 work was begun on the crossing of certain varieties of forcing lettuce. When the plants came into flower it was at once apparent that the problem of emasculation was a knotty one. The flower heads are small and the florets themselves will scarcely bear handling because of their very fragile nature. In one or two instances the stamens were removed, but always with sufficient injury to the remaining parts of the flower to cause it to wither. A flower of lettuce is in fact about as difficult to manipulate by the usual methods of emasculation as it is possible to conceive.

When the flower head expands, the anthers have already dehisced, and the unexpanded stigmas are covered with pollen. (See Pl. I, fig. 1.) The stigmas begin to expand at the tips, and simultaneously masses of pollen fall on the inner surfaces, to which the pollen closely adheres.

In the bud stage the parts of the floret are so easily damaged that endeavors along this line of attack were quickly abandoned. Having read somewhere that pollen grains would adhere readily to the end of a piece of sealing wax previously rubbed briskly, wax was tried, but without success. The idea then suggested itself that the pollen might be successfully removed with a dampened camel's-hair brush. This plan was also a failure, because with the operation ever so carefully performed some of the pollen grains were left on the stigmas.

Success finally came, not with the aid of a camel's-hair brush or sealing wax, but with the aid of the garden hose. A robust plant of the Grand Rapids variety of lettuce opened 15 flowers one morning, and within as many minutes every flower was successfully depollinated. The metal attachment on the end of the hose was cut off; a piece of hose of smaller diameter was placed in the end of the other hose; then a piece of soft rubber tubing of small diameter was placed inside the second piece of hose and the water turned on just enough to do a little more than trickle. By squeezing the end of the rubber tubing (see Pl. I, fig. 2) a very tiny jet of water was secured. This was trained on the lettuce flowers, and perfect depollination resulted.

After the tiny jets of water had played on each flower head for a few seconds not a trace of pollen was to be found and the pistils stood out from the ligules strong and unharmed. Small pieces of blotting paper freely applied to the florets edgewise soon absorbed all of the water. Pollen from the flower of another variety was then applied. In each of the 15 heads of flowers, seeds matured, and all of the resulting seedlings proved to be intermediate between the two parents.

In depollinating, the flower head is held securely between the thumb and the first and second fingers, and in pollinating it is similarly held. In applying the pollen to the stigmas one head of flowers from the pollen bearer is used, or more than one if sufficient pollen is not deposited on the stigmas. Before applying the pollen a few or all of the ligules should be cut off from the pollen-bearing head of flowers, leaving only the pollen-covered stigmas, and the remaining part of the head applied to the flowers which have been depollinated, working it among the stigmas with a circular motion.

In pollinating lettuce flowers and those of other genera in this division of the Compositæ there is not the same necessity for depositing the pollen on the stigma of each floret as there is on the flowers of the plants of the other divisions, because the lettuce flower heads close very soon after pollination. This act of closing will almost certainly cause the slender stigmas to become well covered with pollen because, in the act of pollinating, the ligules are apt to harbor many grains of pollen.

The first cross effected was between the varieties of lettuce known as Grand Rapids and Golden Queen, the former being the seed bearer. A short account of the subsequent behavior of the seedlings may be of interest here as a guide to similar experiments in the future.

The crossing was commenced about the beginning of July. From the application of pollen to the ripening of the seed, sixteen days elapsed, the time varying slightly according to the condition of the weather—warm, dry weather accelerating the ripening period.

The seeds were sown in the latter part of December in order to make certain that the seedlings during the later stages of growth would have the benefit of warm weather and thus insure seed production.

Seeds of each parent were also sown separately for comparison with the crosses in all stages of their growth. Even in the cotyledon stage differences were noted between the cotyledons of the crossed seeds and those of the parents; they were uniformly not as light in color as those of the Grand Rapids, neither were they as deep a yellow as those of the Golden Queen, but any doubt arising as to the differences in color, size, and form of the cotyledons of the crosses compared with those of the parents was dispelled as soon as the first character leaves appeared in the crosses. The first leaf of the cross Grand Rapids × Golden Queen was similar in every respect to the first leaf of the cross Golden Queen × Grand Rapids, thus proving beyond a doubt at that early stage that the mere act of crossing had been a success. The plants of the two crosses could not have been distinguished, so similar were they in appearance.

A large-sized Grand Rapids lettuce, but more yellowish in appearance than that variety, with the leaves slightly less crumpled and the margin less fringed, would be a fairly accurate description applicable to both lots of plants of the first-generation crosses.

Seeds harvested from each of the plants were labeled and sown separately. In the second generation the results were rather bewildering. The seeds from each plant of the first generation gave approximately 30 distinct forms, and out of the entire number of seedlings of the different numbers about 60 distinct forms were noted. Forty-five plants of these were selected for further testing, and it so happened that the plant numbered 39, selected as being the best in the field, gave the most promising progeny when sown indoors. Several were then selected for fixing. Four distinct forms have come true from seed in 1908. The fifth and last of the heading varieties up to that time had about 15 per cent of seedlings resembling the Grand Rapids variety. By selecting seed from each of 36 of the best heading individuals and sowing separately, about two-thirds of the lots have come true. These will be tested still further before being sent out for trial.

TOOLS REQUIRED FOR DEPOLLINATION AND EMASCULATION.

A small pair of scissors is necessary to remove parts of corollas, stamens, etc., of the different flowers. Those shown in figure 1, *A*, are more convenient for use on many subjects than the common scissors, as they can very readily be adjusted to the various needs with one hand. A medium-sized pair of the ordinary kind of scissors used by plant breeders, with blades 1 inch in length, figure 1, *D*, is also necessary.

Forceps are indispensable tools. Those forceps in common use would be better adapted to the needs of the plant breeder were they supplied with a flattened pin attached to the handle (see fig. 1, *C*). This would often prevent the necessity of laying down one tool to take up another while the operator is performing a delicate piece of work and while the eyes are perhaps fixed on a very small object. A needle can be tied on very easily and the combination is an exceedingly useful one. Several pairs of forceps which are self-closing (shown in fig. 1, *B*) are necessary in such lines of work as pollinating depollinated stigmas of alfalfas and many other small flowers. These forceps enable the operator to hold a stamen or a sexual column of a small legume while pollinating. It is difficult to perform this work satisfactorily with the fingers.

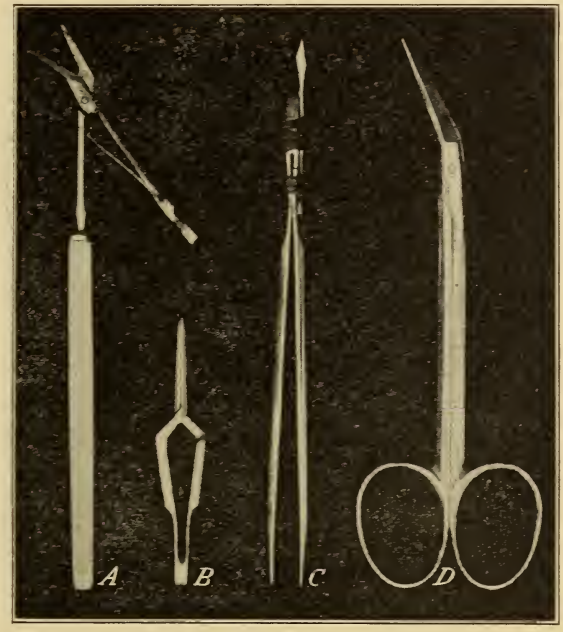

FIG. 1.—Tools used in depollination and emasculation: *A*, scissors useful in removing small organs; *B*, self-closing forceps; *C*, forceps with attachment; *D*, scissors for severing large organs.

Watch glasses and small-sized moist chambers are needed to preserve pollen. Tags for keeping records while the seeds are ripening, ranging from one-half inch by three-quarters inch to those of much larger dimensions, are indispensable. Some small-sized camel's-hair brushes, some good white blotting paper torn into small pieces, and a lens should also be included in the kit.

DEVICES USED IN DEPOLLINATING FLOWERS.

The contrivances required for the work of depollination by water are inexpensive and easily secured. When the method was first used attachments to the garden hose were employed, but these were found too clumsy and uncertain; moreover, the hose is not always available when it is desired to depollinate flowers in the field. A fairly good substitute is a modification of the common putty bulb (fig. 2, *E*). This answers the purpose very satisfactorily for large flowers of the Compositæ, Leguminosæ, and other groups. The putty bulb will hold about a pint of water. By unscrewing the spout or ejector, the rubber bag may be quickly filled with water and the ejector replaced in a few seconds. The size of the jet of water is regulated by using a suitable piece of bamboo reed or other contrivance fixed firmly in the tube. When the bulb is full of water a slight pressure with the hand will cause a fairly strong but fine stream of water to be emitted.

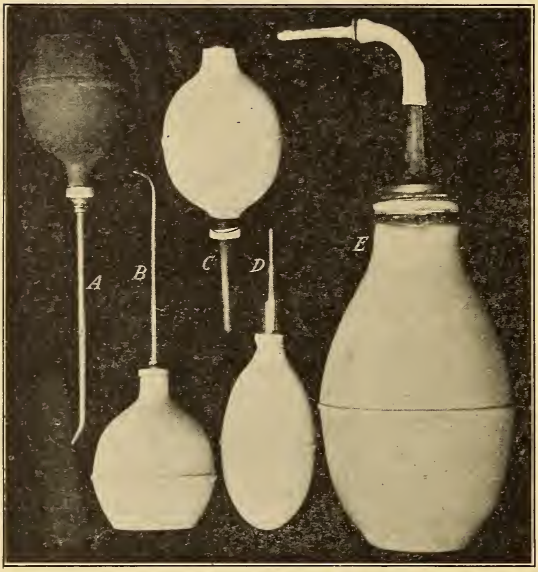

FIG. 2.—Devices used in the depollination of flowers: *A* and *B*, chip blowers or water bulbs; *C*, water bulb with valve at bottom provided with celluloid ejector; *D*, old rubber bulb with glass tube inserted; *E*, "putty bulb" with attachment to give a small jet of water.

It will be found that a very small jet of water is needed for very small and fragile flowers. For this purpose the various kinds of chip blowers (fig. 2, *A* and *B*) and water bulbs sold by dental supply companies suit the plant breeder's needs admirably. These devices are inexpensive, and when many flowers are to be treated at one time several of the chip blowers or water bulbs may be brought into use. As soon as the contents of one are exhausted and while the bulb is still pressed in the hand it may be put in a vessel of water; then when the others are emptied of their contents the first one will be ready to be used again.

There is a still further choice in instruments of this nature in the shape of rubber bulbs (fig. 2, *C* and *D*) similar to those used on cameras.

A piece of glass tubing can be pushed into one of these; the other end of the tube can be brought to a fine point by heating it in a flame and breaking off the point, leaving an aperture about one-sixteenth inch in diameter. This device does satisfactory work, but the others are preferable.

EMASCULATION.

A necessary preliminary to successful plant breeding by hybridization or cross-fertilization consists in preventing pollen of the plant used as the seed bearer, or pollen from other plants of the same variety or species, or pollen of any other closely allied variety or species from gaining access to the stigmatic surface of the flower intended to be pollinated instead of the pollen selected by the operator.

With the greater number of plants, especially those having stamens of large size, emasculation is a simple matter, and consists of removing the stamens before the anthers shed their pollen. Examples of plants having large stamens are found in the rose (see Pl. XI, fig. 1), cherry, peach, etc. The poppy, carnation, and tobacco are also familiar examples in which emasculation is an exceedingly easy process, as the stamens can be removed before dehiscing with a pair of forceps.

OBSTACLES IN EMASCULATING THE FLOWERS OF COMPOSITÆ.

It is well known that the florets of the various genera of the composite flowers are so constructed that the anthers can not be removed without the florets being injured beyond recovery.

The anthers in the disk florets unite and form a tube (Pl. VIII, fig. 1, *B*, and Pl. IX, fig. 1, *B*) over and around the upper part of the immature pistil so that in the effort to remove this tube before the floret opens or the anthers dehisce the anthers are certain to be ruptured and the pollen scattered over the stigma. The parts of the florets are so small that it is practically impossible to remove the anthers in a young stage without ruining the florets. There is also great danger of injuring the very slender pistils during the attempted emasculation. Some genera have only female organs in the ray florets and both male and female in the disk florets, and in some important genera some species have sterile ray florets and depend on the disk florets alone for fertilization. (Pl. V, figs. 1 and 2.) But to depend on the ray florets alone for careful crossing means in all instances slipshod work, as the disk florets must then all be removed, causing too much mutilation of the flower head. Depollination of the disk florets by the method herein described (Pl. VIII, fig. 2, *B*) means that the work is absolutely certain to produce the desired results. Moreover, depollination of the disk florets followed by crossing is many times easier than the use of the ray florets as seed bearers, as this is

necessarily followed by the removal of the disk florets to prevent self-pollination.

In several genera of the Compositæ it will be found that the staminal tubes are not drawn down into the tubular corolla until the second day after shedding the pollen. This happens with some of the species of Helianthus (Pl. IX, fig. 1, *B*), Gaillardia, etc. A short, sharp needle fixed in the end of a piece of wood less than the diameter of a pencil and 4 inches in length can be very successfully used in opening the collar formed by the united anthers around the stigma, so that it may be depollinated before the pollen grains germinate. All of the crop of florets which come out in any single day can be opened after the manner described, the pollen washed from them, and the flower head bagged, pollination being delayed until the following day, when the circles of florets contiguous to those already depollinated will have come to maturity. These are in turn treated with the needle and depollinated by water. The remaining unopened florets may then be pulled out with a pair of forceps, the two lots of treated stigmas pollinated at the same time, and the flower head bagged. A method still easier is to depollinate the two outer circles of stigmas and then remove them with the aid of a pair of forceps, which in the larger number of genera is easy of accomplishment. When the remainder of the florets expand, depollination is effected and the flowers are pollinated as soon as the flower head is cleared of water (Pl. VII, figs. 1 and 2). In all composite flowers which attract insects the head should be bagged to prevent undesirable pollen from gaining access to the stigmas after being pollinated.

It may be said that unless the method of depollination herein described is used with flowers of the Compositæ, especially those of the division Ligulifloræ, there is very little chance, if any, of absolutely certain results from cross-fertilization between different varieties of the same species or from hybridizing distinct species.

HOW HYBRIDS AND CROSSES OF COMPOSITE FLOWERS MAY HAVE ORIGINATED IN THE PAST.

It is quite possible that a long continuous shower will wash the pollen from the stigmas of a composite flower and that as soon as the sun shines these flowers are visited by insects whose bodies are occasionally covered with pollen obtained from florets of other varieties or species which open and shed their pollen, either before or subsequent to the pollen-removing shower of rain. It is certain that cross-fertilization may take place in this way. Those species and their varieties having infertile ray florets can be crossed only when the disk florets, containing both male and female organs, are used as the seed bearers. (Pl. VII, figs. 1 and 2.) All of the work in breeding compos-

e flowers is rendered simple by the depollinating method. Especially is this the case with chrysanthemums, asters, dahlias (Pl. VI, fig. 2), marigolds, cineraria (Pl. VI, fig. 1), cosmos, zinnias, lettuce, and with all the numerous genera having infertile ray florets.

PREPARATION OF SEED AND POLLEN BEARING PARENTS.

Too much care can not be bestowed on isolating from insects the plants from which pollen is to be selected for use in crossing. This is a part of the plant breeder's work which is apt to be neglected. Pollen is usually considered satisfactory irrespective of the conditions under which the flowers have been produced. It is not unnaturally supposed that the pollen is pure while the flowers are in the bud stage, and although this is the case in many instances, especially where large numbers of one variety or species are growing side by side, yet there is no doubt that the pollen of these plants is often interchanged, as in alfalfa and other plants of the legume family in which the anthers dehisce in the flower bud and which are visited by the pollen-eating thrips.

Much experimental work in plant breeding by crossing and hybridization gives negative results through lack of care in the selection of pure pollen. It is just as necessary to protect the flower selected to supply the pollen, in order to prevent foreign pollen from being deposited on or near the anthers either by insects or by wind, as it is to protect the seed-bearing flowers. In selecting pure pollen it is a good plan to have isolated plants growing in pots in the greenhouse, where they can be protected from insects during the flowering period by wire screens. If this is not feasible, the flowers should be bagged when the buds are nearing the opening stage, to prevent insects from depositing pollen on or near the flowers. Absolute success means careful attention to the very minutest detail. Omission of the necessary care in this respect has undoubtedly caused a vast amount of work to be unproductive of good results. Not only should the pollen bearer be grown under glass, but in every case where it is possible the seed-bearing parent should also be grown in this manner in order to have the work absolutely under control. Even then with the aid of wire screens only the large insects can be kept from the flowers as the pollen-eating thrips found in nearly all flowers is one of the greatest carriers of pollen from flower to flower. It is not unusual to find one of these minute insects with several grains of pollen attached to its body, rendering the work of evidently careful emasculation of no avail. The plants infested with these insects should be treated to a slight fumigation with hydrocyanic-acid gas and afterwards protected with structures covered with some kind of fine white fabric to prevent the flowers being visited immediately before emasculation and until fertilization takes place.

CONDITION OF THE STIGMA AT THE TIME OF POLLINATION.

It has often been stated that the stigmatic surface of the pistil must be free from moisture when the pollen is applied. When the contrary condition is present, the pollen is said to be less effective than it would be were it applied to the stigma when free from moisture deposited from the atmosphere. Be this as it may, it is absolutely certain that pollen is as effective when applied to stigmas which have been thoroughly treated with water and the moisture adhering to them partly removed with the aid of bibulous paper applied edgewise as it is when the stigma has not come in contact with water.

THE APPLICATION OF WATER TO ALL FLOWERS.

For the removal of pollen which may have been deposited upon the stigma of a flower previous to pollinating, water should be used in every case where the flower has been exposed through inadvertence or otherwise to the visits of insects. Its use in such a case, if it be applied within a certain period after the pollen is deposited, is desirable, as it will render the operation of crossing with another flower more certain, because if pollen is present on the stigma of a flower, especially if the pollen be of the same variety or species as the flower which it is desired to use as the seed bearer, its own pollen will in many cases take effect in preference to the pollen of the flower of a separate species or variety.

Another case may be mentioned in which this adjunct to perfect emasculation can be used advantageously. It sometimes happens that an operator comes across an open flower of some kind which he may wish to cross, with the stamens already dehisced and the stigmas well covered with pollen. If the pollen has been deposited on the stigmas for only a short time, in most flowers every grain can be removed effectively by the aid of the depollinating method herein described. In the case of lettuce the flowers have been depollinated after the pollen had been in contact with the stigma for an hour and a half and a successful cross has followed.

CROSSING ALFALFA.

WORK ACCOMPLISHED IN THE PAST.

For the purpose of demonstrating how the new method of preparing flowers for crossing can be applied to a wide range of subjects, alfalfa may be taken as an example. While it is true that alfalfa crosses and hybrids are effected by insects, the genus is an exceedingly difficult one to deal with by ordinary methods of emasculation, and there is probably no record of intentional crosses among the many

forms of *Medicago sativa*[a] or hybrids between it and other species, if we except those of Urban,[b] who succeeded in making reciprocal crosses with *Medicago sativa* and *M. falcata* in 1877. The work of Urban, however, was evidently done without taking the necessary precautions to preclude the possibility of error, as the pollination was a crude imitation of that effected by insects.

We can not afford to relegate the crossing of the varieties of this increasingly important plant to insects or to risk pollen of unknown or undesirable forms on the plants we wish to cross. If we were to copy the insect method, we should, of course, get crosses, but we should remain as much in the dark concerning the parentage of the seedlings as we have been in the past.

PECULIAR FEATURES OF THE WORK.

None of the species and varieties of alfalfa sets seed from self-pollination if the flowers remain untripped (Pl. II, fig. 1, *A*). The pollen in the untripped flower, being of a slightly adhesive nature, does not get an opportunity to move after being discharged from the anthers while still within the closed keel, and after the dehiscing period the surface of the stigma is protected from it by being close against the keel. This is the case with the flowers of most of the varieties, but there are forms the flowers of which often have the stigmas completely hidden by the pollen (see Pl. III, *C*), and yet even these do not set seed if the flowers remain untripped.

When, however, the flower is tripped (Pl. II, fig. 1, *B*), either by drawing the closed hand along the raceme or by snipping the individual flowers with the forceps, this action releases the column from its imprisonment within the keel and permits it to spring upward with a very rapid movement. In doing so the pollen grains are thrown on the banner in large numbers and the stigma falls with force among them, causing a mass of the grains to be embedded in it (Pl. II, fig. 1, *B*). This action usually results in fertilization.

When the flowers are visited by certain insects, the upper part of the sexual column falls with considerable force on the under part of the insect. The stigma in this case is then partly pollinated with pollen from the same flower, from other flowers on the same plant, or from flowers of other plants of the same variety or from other varieties, or from pollen of other species previously deposited on the body of the insect.

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[a] The botanical history and nomenclature of this species have been discussed in previous publications of the Bureau of Plant Industry; by C. S. Scofield in Bulletin 131, part 2, "The Botanical History and Classification of Alfalfa," and by Charles J. Brand in Bulletin 118, "Peruvian Alfalfa: A New Long-Season Variety for the Southwest."

[b] Urban, I. Verhandlungen des Botanischen Vereins der Provinz Brandenburg, vol. 19, p. 125. 1877.

OLD METHODS OF CROSSING ALFALFA.

Perhaps the method first used in crossing alfalfa, at least so far as can be ascertained, consisted in introducing a sharpened piece of wood, resembling in shape the proboscis of a bumblebee, into the suture formed by the blades of the keel of the pollen bearer. The piece of wood was dusted over with pollen secured by allowing a sexual column to trip on it. It was pushed into the suture of the flower of the proposed seed bearer, and the resulting tripping of the column caused the stigma to come in contact with the pollen already secured on the piece of wood, the supposition being that, in some cases at least, foreign pollen is prepotent over that of the seed bearer on its own flowers.

The second method—one which the writer tried several years ago—consists in emasculating the flowers in the bud stage, but as the anthers dehisce before the buds expand the operation must be performed when the buds are quite small, and the danger of bruising the flower enough to cause it to wither is great. This operation is easily performed with the aid of a binocular dissecting microscope, but even when the parts of the flower are left uninjured the method is clumsy and exceedingly uncertain.

Another method which involves a considerable element of uncertainty consists in planting one or more plants of a known variety in the midst of a large field of another variety. These plants are used as seed bearers and the work of pollination is left to the insects.

Thus it will be seen that when a cross of known ancestry is desired between two varieties of alfalfa the chances of securing it by methods heretofore in vogue are very remote.

NEW METHODS DEVISED BY THE WRITER.

A few years ago, while investigating methods of crossing alfalfa and trying to cross a hardy form of Peruvian alfalfa with a variety from Turkestan, it was found that there are at least three variations in the method of compelling this plant to capitulate readily to the wishes of the plant breeder.

DEPOLLINATION BY WATER.

The first method to be described requires close attention to details. The tools and other necessary material are as follows: Four pairs of forceps, three of them self-closing; a pair of scissors (see fig. 1, *A*); a few pins; a small chip blower such as dentists use (see fig. 2, *A* and *B*); a vessel of water; and some pieces of good blotting paper.

The operations of depollinating and applying pollen to the stigma can be performed satisfactorily with the unaided eye, but the operator should take his first lesson with the aid of a low-power binocular

dissecting microscope. The magnification should not be higher than 8 diameters.

Three or four flowers on a raceme should be selected for crossing. The others may be cut off, although this is not necessary if they be tripped and the surplus pollen removed by washing with water. However, when all but three of the flowers on a raceme are removed, those intended for crossing can be reached more easily. The age of the flowers used as seed bearers for crossing seems to make little difference, provided they do not show signs of withering. It is perhaps the safer plan to select those flowers near the center of the raceme just when the buds at the end of the raceme are about to expand. The flowers should not be mutilated in any way, and of course should be handled as little as possible because of their delicate nature.

The first requisite consists in having pollen from the male parent at hand ready to be applied to the prepared stigmas. To do this most conveniently, as the flowers can not very well be manipulated with the fingers alone, it is desirable to have three pairs of forceps, one for each flower that is to be pollinated. Self-closing forceps are best, because they hold firmly the sexual column (Pl. III, *C* and *D*) used in pollinating. Take a flower from a raceme of the male parent, bend down and secure the banner between the tips of the thumb and the forefinger, then press with a pin or dissecting needle sidewise against the suture of the keel, beginning at the base and gradually drawing upward. If this operation is done carefully, the column will come out gently without disturbing the pollen from the anthers. When the flower has been tripped or exploded in this way, the terminal part comprising the stigma, with the masses of pollen surrounding the empty anthers closely arranged around it, hangs toward the operator free from contact with anything. With the aid of the self-closing forceps, sever the column from the flower, laying the columns aside ready to be applied to the stigmas of the flowers of the seed bearer when the latter is depollinated.

Now comes the most critical part of the operation, and the amount of skill with which it is performed determines the success or failure of the work. It must be understood that in the flower to be used as the seed bearer the stamens dehisced while the flower was in the bud stage, perhaps a day or two previously, and the pollen lies in masses all around the soft stigma (Pl. III, *C* and *D*), but still incompetent in that position to perform the acts of pollination and fertilization while the column is untripped. It should be the aim of the operator to trip the column in a manner that will cause a minimum disturbance of the arrangement of the pollen and prevent the terminal part of the column containing the sexual organs from springing with considerable force on the awaiting banner, thereby embedding a large number of its own pollen grains in the soft, pulpy stigma.

With this end in view, grasp one of the flowers gently but securely between the tips of the thumb and forefinger, with the back of the keel resting against the tip of the index finger. Cut an ordinary small-sized pin or needle in two, take the pointed end between the thumb and index finger of the free hand, place the half pin or needle against the lower part of the suture of the keel, and press gently against the keel, bringing the pin or needle up to the central part or a little beyond it, increasing the pressure gradually. This will compel the sexual column to alter its position, or "trip," gradually as the gentle pressure of the pin retards or prevents its springing with force enough to disturb the arrangement of the pollen grains around the stigma. Allow the pin supporting the sexual column to come gently toward the awaiting banner. It will then be found that, with the pin resting on the banner, the fingers may relax their hold, the pressure of the column toward the banner keeping the pin in place; and owing to the position of the pin, for the time being the terminal part of the sexual column, consisting of stigma and an abundance of pollen around it, is prevented from pressing on the standard, as it is about one thirty-second to one-sixteenth of an inch above it. (Pl. II, fig. 1, *C*, and Pl. II, fig. 2.) This gives the opportunity for the removal of the pollen grains by the use of a jet of water from the chip blower.

The action of the water effectually depollinates the flower without causing the least injury (Pl. II, fig. 1, *D*); in fact, the jet may be of sufficient force to remove even the empty anthers without injury to the stigma. (Pl. III, *E* and *F*.) However, the first few flowers operated on by the beginner should be examined before proceeding with the pollination to ascertain if the treatment given has been sufficient to depollinate the flower thoroughly.

After the jet of water has been applied there will be a considerable quantity of moisture covering the different parts of the flower, especially the empty anthers and stigma. This is immediately removed by touching these organs and other parts of the flower with a piece of blotting paper applied edgewise.

When this operation has been completed the exposed stigmas are pollinated in the following manner: Take one of the self-closing forceps, holding one of the previously prepared sexual columns from the flowers of the male parent, and with the stigma pointing upward push the end of the column containing stamens and freed pollen closely surrounding the stigma under the end of the column which has been depollinated, giving it a very slight circular movement to make certain that the large masses of pollen come in contact with the soft stigma of the depollinated flower. When this has been satisfactorily done, take hold of the supporting pin by the blunt end and gently withdraw it; the column then assumes its place on the banner

with the stigma closely pressed against its surface (Pl. II, fig. 1, *F*), and a goodly number of pollen grains are embedded in the soft stigmatic surface. When the supply of pollen is unlimited a number of flowers may be tripped over a watch glass and the pollen applied to the stigma with a small brush, the hairs of which are held together with a weak sirup of sugar and water.

If the details described are carried out in a painstaking way, all that is done simply consists in substituting pollen from another variety for that which originally surrounded the stigma of the flower of the proposed seed bearer.

The operation is performed in much less time than it takes to describe it, and the operator is rewarded by a fairly high percentage of successful crosses. The first time the writer tried this method with two distinct varieties of *Medicago sativa* more than two-thirds of the flowers worked set seeds.

The second method of crossing alfalfa also involves depollination with the aid of a jet of water on the dehisced stamens, but it has now been discarded in favor of that just described. It is a simpler operation, requiring less delicate manipulation, but the percentage of successful crosses by it is very low. Besides, it involves the mutilation of the floral envelope and the exposure of the pollinated stigma to the atmosphere instead of allowing it to assume its natural position on the banner of the flower after being pollinated.

By this method all the flowers on a raceme may be used. First, by the aid of the scissors shown in figure 1, *A*, cut off all of the unopened buds and the banner of each flower left on the rachis. The reason for removing the banner is that when the flower column is tripped the position of the sexual column is altered so as to free it from contact with anything and to render it readily accessible.

The depollination of the flower thus becomes a very simple problem. The sexual column, being still imprisoned within the keel, is best tripped by a very light snip given by a pair of forceps, care being taken to place the tips of the forceps at a point near the base of the column so that the stamens and pistil are not interfered with in withdrawing. The sexual column being now free from the keel, the latter, together with the wings, should be cut off.

A small jet of water is then trained on the sexual organs from a water bulb or chip blower (see fig. 2, *A* and *B*); the stream should be quite gentle at first, followed with just sufficient force to remove both pollen and empty anthers. This will facilitate an easy approach to the stigmas when pollinating. Examine the stigmas with the aid of a lens to ascertain if all of the pollen has been removed; then remove the surplus water by applying lengthwise a piece of thick and very bibulous blotting paper. The flowers are now ready to be pollinated.

In preparing the sexual column of the pollen-bearing flower so that the pollen may be easily and quickly applied to the stigmas of the depollinated flowers of the seed bearer, detach one flower at a time and pollinate as described for the first method of crossing. If the pollinated stigmas are left exposed to the air, fertilization takes place only in a very small percentage of the flowers. They give a higher percentage when protected by a small paper bag, or each raceme may be placed inside of a test tube and kept in place by a small wad of cotton for twenty-four hours after pollination, the test tube being tied to a support and shaded from direct sunlight.

DEPOLLINATION BY COMPRESSED AIR.

The third method by which alfalfa and other flowers have been successfully depollinated is the use of compressed air. Contrary to expectation, this has no injurious effect on the tender parts of the pistil.

The parts of the flower are prepared in a manner similar to that described in the first method. The current of air is obtained from a small cylinder into which air is pumped to an indicated pressure of 20 pounds. The nozzle of the air tube is held about half an inch from the terminal part of the column by a helper; the full force is turned on just when the column is about to spring from the keel and is continued for ten or fifteen seconds after resting on the pin.

Pollination and subsequent treatment are performed in the same manner as given for the first method.

Fertilization following this method is satisfactory, but the percentage of successful crosses is not as high as in the first method described.

In depollinating large flowers of other genera having pistils that may be easily injured, it is very helpful to remove large quantities of pollen previous to the more thorough work by a jet of water from the chip blower.

THE GROWING OF THE PLANTS AND THEIR CARE.

In order to have the parent plants of alfalfa intended for crossing thoroughly under control, they should first be raised from seeds to ascertain if the varieties come true. The plants to be crossed should be grown in a cool greenhouse and when of sufficient size put in large pots, as then they are less apt to become suddenly dry at the roots. Good fibrous loam and a little rough sand make a good soil for pot culture. To prevent souring of the soil, place some large potsherds over the drainage hole, and over these place some half-rotted leaves. Ram the soil moderately firm around the roots, leaving sufficient space to give enough water at a time to last for two or three days.

It should be the aim of the cultivator to have the plants in bloom about the end of March or the beginning of April. At that season the absence of insects will render the work easier of accomplishment than when grown outdoors. Under outdoor conditions the plants would require to be protected by wire or cloth screens to exclude pollinating insects. Several specimens of each variety to be worked with should be grown to make certain of having some of them in bloom at the proper time. A strong plant of the variety to be used as the seed bearer should be selected.

RAISING LARGE QUANTITIES OF SEED FROM A CROSS.

When a promising variety of alfalfa has been secured by crossing, the problem of how best to secure a quantity of seed to sow a large area is undoubtedly a serious one. In the first place, the plants should be tested thoroughly to ascertain if they are superior to existing forms. The crosses obtained so far do not seem to vary much in the second and succeeding generations when seed is saved from flowers tripped by the hand. Those that prove of value from a single cross, the individuals of which are evidently alike, should be propagated vegetatively and the progeny of each plant kept separate till planted out in the field, when they may be mixed for cross-pollination. The cuttings will root satisfactorily in a cold frame if kept closed for a few days. It is possible by this method to root several hundred cuttings during the summer, beginning with a single mother plant in the spring, but it must be understood that these cuttings originating from a single mother are to be considered as one plant; that is, if seeds set poorly on the mother plant as a result of self-pollination by artificial tripping, all the plants propagated asexually from the original will have the same peculiarity. If the asexually propagated progeny be planted in a field a safe distance from other varieties, the probabilities are that the plants from the resulting seed will come true and the strain be established. In this way a much larger crop of seed can be secured in a given time than if one depends altogether upon seeding the original individuals of a cross.

CROSSING LARGE-FLOWERED LEGUMES.

In crossing varieties of legumes which come true from seed resulting from self-pollination, it is not necessary to work with a large number of flowers. Careful manipulation of a few will give all the possible results with any two varieties, and usually the operator will get more varieties than he desires in the second and subsequent generations. The flowers of such plants as Phaseolus, Stizolobium, Vigna (Pl. IV, fig. 1), Pisum, and Lathyrus should be emasculated in the bud stage and before the anthers shed their pollen. The operator before under-

taking to cross two varieties should have a perfect knowledge of the parts of the flower.

The idea of having large models of the various flowers of the principal garden and field crops in agricultural colleges and schools is a good one. It enables prospective plant breeders to become familiar with the structure of the common flowers and shows what is necessary to be done in preparing flowers for crossing more quickly than the study of the flowers themselves or the use of illustrations made by others. Many of the flowers of forage plants and vegetables are so minute that it is with difficulty that they are emasculated even with the aid of a good dissecting microscope. This is the case with all the species of Melilotus and a goodly number of the species of Medicago and Trifolium; and the student, having the forms and structure of the flowers continually in his mind, will be better prepared to cope successfully with plant-breeding problems.

Among the large-flowered legumes, varieties of which it is desired to cross, the cowpea may be chosen as a good example in order to show how the flower is manipulated previous to pollinating. In the evening it is found that the buds which will expand the next morning are quite large and easily manipulated in emasculating. (Pl. IV, fig. 1, *A*.) Hold the bud between the thumb and the forefinger, with the keeled side uppermost (Pl. IV, fig. 1, *B*); then run a needle along the ridge where the two edges of the standard unite. Bring down one side of the standard, securing it in position with the thumb; then do the same, with one of the wings, which will leave the keel exposed. This must be slit on the exposed side about one-eighth of an inch below the bend in the keel and continuing along until about one-sixteenth of an inch from the stigma, which can be seen through the tissue of the keel. Bring down the section of keel and secure it under the end of the thumb. This will expose the immature stamens, 10 in number. (Pl. IV, fig. 1, *B*.) With a fine-pointed pair of forceps seize the filaments of the stamens and pull them out, counting them as they are removed to make certain that none are left. (Pl. IV, fig. 1, *C*.) Allow the disturbed parts of keel, wings, and standard to assume their original positions as far as possible. Next detach a leaflet from the plant, fold it once, place it over the emasculated flower bud, and secure it in position with a pin or toothpick. This will prevent the bud from drying out before the stigma matures sufficiently to be pollinated from a flower of a different variety next morning. If the stamens are removed without altering the position of the pistil (Pl. IV, fig. 1, *C*) or injuring it in any way and pollen is applied the morning after the flower is emasculated (Pl. IV, fig. 1, *D*), in nine cases out of ten they will set seed; and if none of the anthers were ruptured in their removal, the resulting

seedlings will have some of the characters of both parents. If the two parents have spotted seeds and the seed bearer, for instance, be the well-known variety New Era (Pl. IV, fig. 2, *A*) and the pollen bearer the equally well-known Whippoorwill variety (Pl. IV, fig. 2, *B*), the seeds resulting from the first-generation plants will have the markings of both parents on each seed, giving a beautiful example of crossing in the seed itself. (Pl. IV, fig. 2, *C*.) In the second generation we have a strange combination. About nine parts of the resulting seeds are like those of the first generation, three parts like Whippoorwill, three parts like New Era, and one part self-colored, resembling the lighter or ground color of the straight Whippoorwill seeds; but in the first three lots, although the series are easily classed, there are upward of 30 variations in color alone, and many more when we take form and size into consideration. In subsequent generations they again vary until by following defined rules we get new fixed types.

However, the variations in cowpea crosses are not always so very apparent, especially when two varieties having seeds of the same color are selected as parents. While there are great differences in the other characters of the progeny, such as foliage, early and late ripening, vining habit, upright growth, and disease resistance, the seeds differ from the parents and among themselves apparently only in size and shape; and while in the first-mentioned cross one could pick out dozens of dissimilar seeds in the colors alone, three or four variations in size and shape are about all that the operator may expect from crossing varieties the seeds of which are similar in color but vary in size.

In the condition of the mother plant at the time of pollination there is fortunately some choice which makes for a high percentage of successful pollinations. It will be found, especially with the cowpea, that young plants allowed to fruit in 5-inch pots set seed with greater certainty than will rampant-growing plants in large pots. Again, old plants in large pots break freely into growth, but not of a viny nature. This growth produces flowers in abundance, and pods form with great freedom either from their own pollen or when crossed with pollen from other varieties.

CROSSING IN LARGE AND SMALL NUMBERS.

A matter of importance in plant breeding and one which does not receive the attention it deserves from the practical breeder is the grouping of varieties into at least two classes for somewhat different treatment in crossing. In the first division all the plants we now call varieties, which come true from seed, no matter how they originated, may be termed artificial species. This division includes

garden and field crops, such as lettuce, cabbage, and turnips; radishes and others of this family; onions and other plants of the same natural order; carrots, celery, parsley, and parsnips; the cereals; varieties of alfalfa, and many others which come true from seed and are only propagated sexually. With these there is no necessity for raising many individuals of the first generation. If the breeder succeeds in getting a desirable cross between two well-defined kinds, a few individuals of the first generation are enough, provided the work of emasculation or depollination and the application of pollen to the flower so treated is not carried out in a perfunctory manner. The individuals of the second generation, if they are sufficiently numerous, will give the variations from which to choose selections for perpetuating.

The second great division includes those plants which do not come true from seed or at least have never been bred to reproduce in that manner. The treatment in crossing is different from that which should be accorded the sexually propagated varieties, in that large numbers of the first generation are necessary, so that the chances of securing improved forms will be greater in proportion to the number of seedlings raised from any one cross. Nearly all fruit and nut trees, grapevines, bush fruits, strawberries, and potatoes are included in this class; also many florists' flowers, such as roses, carnations, gladiolus, dahlia, fuchsia, chrysanthemums, and pelargonium. When the desired improvement is attained by crossing any two varieties, one plant of the improved form is sufficient to start with, and that is increased vegetatively, i. e., by cuttings, budding and grafting, layers, bulblets, cormlets, etc.

In this second division, when breeding improved forms by crossing is attempted we look for results in the first generation; consequently, the number of flowers pollinated must be large, so that the seed ripened will be in quantities large enough to give the necessary variation in the resulting seedlings. The plants mentioned above which are propagated asexually are all hybrids, crosses, or sports which have never been bred sufficiently to come true from seed; thus, when any two plants of distinct varieties are crossed we never can tell exactly what the progeny will be like, and it is as a rule so very varied that if we get one seedling in five thousand possessing characters superior to either parent we are doing well.

HYBRIDIZING SPECIES.

HELIANTHUS HYBRIDS.

It may be necessary at times to hybridize two natural species. If a hybrid is raised, the probabilities are that it will not set seed with its own pollen. This was the case in a hybrid recently raised

between *Helianthus argophyllus* and *H. debilis*, the latter being the pollen-bearing parent. The plants, although subjected to the best treatment, did not set a single seed from their own pollen or from pollen applied from one flower head to the stigmas of another on the same plant; nor was a single seed set on a plant the flowers of which were pollinated with pollen taken from flowers on separate plants; but when a flower of the hybrid was pollinated with pollen from the male parent, seeds were produced freely. These when sown and the plants put in the open ground also seeded very abundantly; the flowers resulting from these seedlings were much finer than those of the parents or any of their forms. It remains to be seen, however, just how the third generation will turn out, as in the seedlings of this generation, now only a few inches high, the variation of the foliage is considerable.

GRASS HYBRIDS.

In hybridizing two species of grass lately the results obtained would indicate, in this instance at least, that large numbers of hybrid seedlings are not necessary in the first generation of grass hybrids. Out of 13 seeds secured, 10 germinated, and no two plants are alike; in fact, the variation in the progeny of the first generation is more marked than in any second-generation seedlings of any other cross between two varieties with which the writer is familiar. The parents were *Poa arachnifera* and *P. pratensis*, the latter being the pollen bearer. The seed bearer was a hermaphrodite plant and the only one out of about 400 seedlings.

The flowers of the seed bearer were thoroughly treated with water each morning until the crop of stamens was exhausted. A very large quantity of pollen from *Poa pratensis* was secured by cutting half an armful of culms of *P. pratensis* when in bloom; these were put in a vessel of water in the evening. Next morning the stigmas of *P. arachnifera* were thoroughly pollinated by taking one handful at a time of the culms of *P. pratensis* and shaking them over the depollinated stigmas of the Texas bluegrass. This was done in a greenhouse; the stigmas were almost hidden by the pollen. Only 13 seeds resulted, but these were more than enough, as each of the progeny might well have been taken for a new species.

Each of the 10 seedlings was propagated by division and 100 plants of each put out in the field. No seeds were produced, as the pollen was imperfect; not a single grain was found in good condition.

Pollen from *Poa pratensis* was again applied in 1908, and the plants seeded abundantly. The resulting seedlings are exceedingly varied. Some have long, broad leaves; others short and broad, narrow and short, or narrow and long leaves.

CROSSING CULTIVATED VARIETIES ON NATURAL SPECIES.

PANSIES.

To show what might be expected in renewing the vigor of some varieties by crossing them on wild progenitors, it may be permissible to mention some work in the improvement of the common pansy in an attempt to enable it to better withstand our hot summers. Some time ago a number of plants of *Viola tricolor* were found growing luxuriantly in southern California in hot and dry places. Even far down in the Imperial Valley, where the temperature frequently reaches a stage unheard of in the East, the plants seemed at home.

The idea suggested itself that this plant might be of use in imparting heat-resistant characters to the cultivated pansies. Plants were raised from the seed gathered and pollen from a strain of pansies applied to the stigmas of the wild plants. Some of the flowers of the first-generation progeny are shown in Plate XIV. The petals were removed from the flowers of the wild plant and their stigmas cleared of pollen with the aid of water previous to pollinating. All the plants secured have fair-sized flowers, more or less resembling in color and markings those of the wild type. The foliage more closely resembles the mother than it does the father. Recrossing with pollen from a good strain the present season will probably give the desired size next season.

DAHLIAS.

The original Twentieth Century dahlia was used in pollinating a new species from Mexico four years ago. This species has very small, bright-red flowers, and the stems are exceedingly long. The larger and very rough leaves are produced near the crown of the plant, leaving the principal stems with only very small leaves. The flowers of the Mexican plant were depollinated (Pl. V, figs. 1 and 2) and pollen applied to three of the flowers. Sixteen seedlings resulted; the small number of seeds was probably due to the fact that the mother plants were growing in small pots in the greenhouse and did not flower till after the outdoor crop had been blackened by frost. The pollen was obtained from flowers which had been saved and kept in water.

The resulting first-generation seedlings were strictly intermediate between the parents, all of them with very long stems (see Pl. XV), but the varieties were not striking enough in color or size of flower to warrant vegetative propagation. The best of them were bagged and allowed to set seeds. The seedlings of the following season gave a very large assortment of forms, and the colors were more varied than those of the first generation. About fifty forms were saved

for further crossing and selection. The following summer the best of these were crossed with pollen from the cactus and other varieties. As a result a few crosses were produced which show that varieties may be raised in this way with large and well-formed flowers, some of them equal in these respects to some of our best varieties bred from plants in cultivation for nearly a century. One of the forms with single flowers is shown in Plate XV.

CROSS-POLLINATING CLOVERS.

To pollinate clover flowers artificially may seem a difficult operation on account of their minute and delicate structure, but in reality it is even more simple than the manipulation of the flowers of alfalfa. Many observations indicate that the flowers of the red clover are incapable of self-fertilization when protected from insects, as plants which have been tested with this end in view have in no case produced seeds. It would, however, seem that the emasculation of clover flowers is unnecessary, because when the keel is pulled forward and the stamens disturbed it rarely happens that the pollen comes in contact with the stigma.

When it is desired to perpetuate well-defined varieties by careful intercrossing of individuals, the work of transferring pollen from the anthers of one plant to the stigmas of another can be rapidly and effectively performed in the following manner: Select plants of both the proposed pollen and seed bearers which have developed flowers under the protection of a wire screen. We can then be reasonably certain of the absence of insect interference. Take a flower of the pollen bearer between the thumb and forefinger of the left hand, and using the forceps having a flattened pin tied to one end, as shown in figure 1, *C*, place the flat side of the pin parallel with the standard of a floret, the pin pointing to the base of the keel, and draw it gently upward. The result will be that many grains of pollen will adhere to the flattened portion of the pin. Turn the forceps in the hand so that the prongs take the position first held by the pin. This must be done carefully so as not to dislodge the pollen on the end of the pin. Then with the prongs of the forceps snip a piece from the end of the banner of the flower intended to be pollinated. This will show which of the flowers on a head have been manipulated. Next take one of the prongs of the forceps and bend down the keel and wings of the floret to be pollinated, securing them in a position under the end of the thumb. This operation will bring the upper part of the pistil into view. The forceps are now turned in the hand to their first position and the pollen carefully applied to the stigma. The operation is concluded by restoring the keel and wings to their original positions.

METHODS OF EMASCULATING AND POLLINATING COMMON FLOWERS.

THE ESSENTIAL ORGANS OF THE FLOWER.

In the greater number of flowering plants there are what are usually termed male and female organs in each flower. The rose (Pl. XI, figs. 1 and 2) and canna (Pl. IX, fig. 2, *A*, *B*, and *C*) are familiar examples. These are called hermaphrodite flowers.

Other plants have the male and female organs in separate flowers, but on the same plant, as in the begonias (Pl. XII, fig. 1) and the genus Codiaeum (Pl. XII, fig. 2). Flowers of this nature are termed monœcious.

There is still another class of plants which has the male flowers on one plant and the female flowers on another. Examples of this arrangement are found in the willows (Pl. XIII, fig. 1), the aucuba, the genus Nepenthes, and commonly in the edible asparagus (Pl. XIII, fig. 2). These flowers are termed diœcious.

The so-called male organs are the stamens (Pl. XI, fig. 1, *Sta*), usually consisting of the filaments, or stalks, and the anthers containing the powdery material, or pollen. The so-called female organ is the pistil (Pl. XI, fig. 1, *P*). The lower part is the ovary (Pl. IX, fig. 2, *a*, *o*); the next part, in some flowers absent, is known as the style (Pl. III, *E* and *F*). The terminal part, that on which the pollen is deposited, is the stigma (Pl. III, *E* and *F;* Pl. IX, fig. 2, *S*).

PREPARATION OF FLOWERS TO BE POLLINATED.

In crossing plants which have both male and female organs present in the same flower (Pl. XI, fig. 1) the principal point to be kept in view is the removal of the anthers from the flower which is chosen as the seed bearer before the pollen is ripe; this is to prevent self-pollination of the flower. It is also necessary to prevent pollen from other flowers on the same plant or from flowers on other plants of the same variety or species gaining access to the stigma of the flower to be cross-pollinated. To this end it is always advisable to have isolated plants for seed bearers.

A few of the flowers or as many as can be conveniently worked at one time may be selected for crossing, and the others removed. The selected flowers should be covered with paper bags, or, better still, the whole plant if not too large may be covered with a small-mesh wire screen, which will effectually prevent pollination by winged insects.

In some plants the anthers of the flower intended to be used as the seed bearer must be removed when the flower is in the bud stage,

as the stamens mature long before the pistil (see Pl. X, fig. 2). This operation in many cases necessitates the mutilation of the petals; therefore, emasculation in the bud stage should never be performed except in those cases where the anthers shed their pollen before the petals expand. Many plants shed their pollen only after the petals expand, as in the rose and the lily, and this is the safest time to remove the anthers provided the flowers have been protected against access of pollen to the stigmas.

In plants like *Tecoma grandiflora* the anthers dehisce long before the flower opens, but the lobes of the stigma are closed and do not open until the anthers of the same flower have shriveled. However, the pollen grains which lie in the tube of the flower might be brought into contact with the stigma; therefore, it is safest to remove the anthers before they open.

With some plants, such as the anthuriums, the stigmas are ripe several days before the pollen matures. In this case the anthers can not be removed, nor is there any necessity for their removal in crossing. The common plantain (*Plantago lanceolata*) (Pl. X, fig. 1) is another plant in which the pistil matures a short time before the stamens of the same flower, thus insuring cross-pollination.

Those plants which have the stigmas and the stamens in separate flowers on the same plant, as in the genus Begonia and the genus Codiaeum (Pl. XII, figs. 1 and 2), should have the staminate flowers removed before they open and the pistillate flowers inclosed in small manila paper bags both before and after pollination, or if the proposed seed bearers are growing in pots they should be isolated and screened.

With plants which have pistillate flowers on one plant and staminate ones on another, as in the genus Nepenthes, willows (Pl. XIII, fig. 1), and asparagus (Pl. XIII, fig. 2), it is only necessary to guard against undesirable pollen by bagging the flowers before and after pollination.

REMOVAL OF THE ANTHERS.

The removal of the anthers in the case of flowers having both stamens and pistils is called emasculation. There are various ways in which the anthers may be removed. Perhaps the best method is to use the forceps shown in figure 1, *C*, as the most delicate stamens may be seized and their anthers removed with this instrument. When all the stamens are visible to the eye the process is an easy one. When, however, the operator wishes to emasculate a flower of a legume (Pl. IV, fig. 1) or of any other plant in the bud stage, he should know the number of stamens in the flower of each species, and the anthers should be counted as they are removed, thus making certain of the complete emasculation of the flower.

SECURING POLLEN.

To have the pollen of a flower to be used in pollinating absolutely pure, it is necessary that the stamens be protected, both before and after dehiscing, by the use of paper bags. To secure pollen in sufficient quantity to make certain that the stigmas of the proposed seed bearer are well covered, the best method is to secure a few flowers just before the anthers open, with stems long enough to go into a vessel of water. Let the vessel containing the flowers stand indoors until the stamens dehisce; then hold that part of the flower on which the stamens are situated over a watch glass and gently agitate the stamens with a pin. The pollen from a few flowers—of the rose, for instance (Pl. XI, fig. 2, *A*)—will fall in sufficient quantity to cover the glass. Then place the glass in a small box so that it may safely be carried from place to place. Some flowers have pollen which can not well be treated in this manner because it clings together in masses, as in the cowpea and the lily.

APPLYING THE POLLEN TO THE STIGMA.

In those flowers having pollen which clings together and adheres to the stamens the pollen is best applied to the stigmas directly from the stamens by means of the flattened pin shown in figure 1, *C*. Pollen such as that of the rose should never be applied with a dry brush unless there is a large quantity available, as this method means the loss of a very large percentage of the grains. In carrying the pollen from the glass to the stigma the least jar or movement of the air causes it to fall from the brush.

The most satisfactory method of transferring dry pollen is to use a very small brush prepared in the following manner: Dip the hairs in a weak sirup of sugar and water, draw them between the finger and thumb to remove the surplus moisture and to flatten the mass of hair, clip off a small portion of the ends so that the hairs will be all of one length, and trim the sides, which will result in the hairs sticking together. While still damp push the end of the flattened brush among the pollen grains, and even the driest pollen will adhere in masses. It is thus abundantly and easily applied to the most delicate stigmas. The experienced breeder can tell at a glance when the stigma of any flower is ready to be pollinated. The stigmas of the rose shortly after the petals expand indicate the receptive condition, and this may be taken as a guide for the stigmas of most flowers.

DESCRIPTION OF PLATES.

PLATE I. Fig. 1.—Staminal tubes and stigmas of lettuce flowers (enlarged 20 diameters): *A*, Staminal tube; *B*, stigma appearing through end of tube; *C*, stigma covered with pollen; *D*, stigma depollinated. Fig. 2.—Depollinating lettuce flowers with water from a garden hose.

PLATE II. Fig. 1.—Flowers of alfalfa (enlarged 5 diameters), showing the method of depollinating and crossing used: *A*, Untripped and unpollinated flower; *B*, tripped and self-pollinated flower; *C*, flower showing sexual column tripped against a pin, to prevent self-pollination and to provide the opportunity for depollination; *D*, depollinated flower; the operation is performed by training a jet of water on the sexual organs while the column is resting on the pin; *E*, flower showing the stigma pollinated with pollen from the flower of a distinct variety or species while the column is still resting on the pin; *F*, flower from which the pin has been withdrawn after pollination, allowing the stigma to press against the surface of the banner. Fig. 2.—Raceme of alfalfa flowers (enlarged 6 diameters). This illustration shows that when the column of the flower is tripped the pressure is sufficient to hold a small pin.

PLATE III. Sexual columns of alfalfa flowers (enlarged 10 diameters), showing different stages of development: *A* and *B*, Columns with anthers just before the dehiscing stage; *C* and *D*, columns with anthers dehisced; *E* and *F*, columns with pollen and empty anthers removed by the aid of water previous to artificial pollination.

PLATE IV. Fig. 1.—Flowers and young pods of the cowpea (twice natural size): *A*, Flower bud showing condition on the evening of the day previous to the opening of the flower; *B*, flower in the bud stage, showing how the floral envelope is opened to gain access to the stamens for emasculation; *C*, flower with stamens removed, showing the large stigma to the left; *D*, emasculated flower the next morning after pollination; *E*, young pod the second morning, showing that fertilization has been accomplished; *F*, the same pod forty-eight hours after the pollination of the flower. Fig. 2.—Seeds of cowpea parents and of a first-generation cross (enlarged $2\frac{1}{2}$ diameters). *A*, New Era; *B*, Whippoorwill; *C*, New Era ♀ × Whippoorwill ♂.

PLATE V. Fig. 1.—*Dahlia chisolmii* (enlarged 4 diameters), showing disk florets before depollination. The stigmas are densely covered with pollen. Fig. 2.—*Dahlia chisolmii* (enlarged 4 diameters), showing disk florets after depollination by a jet of water.

PLATE VI. Fig. 1.—Disk florets of the greenhouse cineraria before depollination (enlarged 5 diameters), showing the stigmas covered with pollen. Fig. 2.—Disk florets of the greenhouse cineraria (enlarged 5 diameters) after depollination with water.

PLATE VII. Fig. 1.—Flower of dahlia (enlarged 5 diameters), showing the disk florets, the stigmas of which are nearly all covered with pollen. The stigmas of the inner florets are not fully developed. On these the pollen is very abundant. Fig. 2.—The dahlia flower (enlarged 5 diameters) shown in figure 1, with the outer florets depollinated by a jet of water. The stigmas of the inner florets are not fully developed.

PLATE VIII. Fig. 1.—Dahlia florets (enlarged 10 diameters), showing development of the stigmas: *A*, Unopened disk floret; *B*, floret after the staminal tube enveloping the stigma has appeared above the corolla; *C*, floret with the stigma partly protruding from the staminal tube; at this stage it is thickly covered with pollen; *D*, floret with the stigma still farther advanced; the upper part of the staminal tube is seen near the base of the stigma; *E*, floret with the stigma fully developed and covered with pollen; *F*, floret with the stigma depollinated with water. Fig. 2.—Dahlia florets (enlarged 6 diameters), before and after depollination: *A*, Disk florets, showing the stigmas covered with pollen; the staminal tubes have been pulled within the corollas; *B*, florets with the stigmas depollinated by water.

PLATE IX. Fig. 1.—Disk florets of Helianthus (enlarged 5 diameters), showing different stages of development: *A*, Floret in bud stage; *B*, floret, showing staminal tube inclosing stigma, the upper part covered with pollen; *C*, floret (twenty-four hours later), showing the stigma covered with pollen and the staminal tube partly drawn within the corolla; *D*, floret with the stigma depollinated; this is done when the floret has reached the stage shown in *B*. Fig. 2.—Flowers of *Canna indica* (natural size), showing stamen and pistil in different stages of development. *Sta*, Stamen; *S*, stigma; *O*, ovary.

PLATE X. Fig. 1.—Flowers (proterogynous) of *Plantago lanceolata*, showing pistils maturing before the stamens: *A*, Flower head, showing mature pistils; *B*, flower head, showing a few stamens to the right; *C*, flower head, showing withered stamens at the base of the flower spike; *D*, flower head in a more advanced stage. Fig. 2.—Flower (proterandrous) of *Campanula rotundifolia* (enlarged 3 diameters), showing stamens maturing before the pistil. *A*, Flower bud; *B*, flower bud with corolla removed, showing large, mature stamens encircling the immature pistil; *C*, flower with portion of corolla removed; the stigma is still immature, the anthers have discharged their pollen, and the stamens are wilted and curled around the base of the style; *D*, flower after the corolla has withered and the stigma has expanded.

PLATE XI. Fig. 1.—Single tea rose, showing how easily hermaphrodite flowers having large stamens may be emasculated: *Sta*, Stamens; *P*, pistils. Fig. 2.—Flowers of roses (natural size), showing method of emasculation: *A*, Flowers with petals removed, showing stamens and pistils; *B*, flowers with stamens removed, showing stigmas ready to be pollinated.

PLATE XII. Fig. 1.—Flowers (monœcious) of begonia, showing the sexes in different flowers on the same plant: *A*, *A*, Pistillate flowers; *B*, *B*, staminate flowers. Fig. 2.—Staminate and pistillate flowers of *Codiaeum variegatum* from the same plant. *A*, Male flowers; *B*, female flowers.

PLATE XIII. Fig. 1.—Staminate and pistillate (diœcious) flowers of willow from different plants: *A*, Female flowers; *B*, male flowers. Fig. 2.—Flowers from staminate and pistillate plants of *Asparagus officinalis* (enlarged 5 diameters): *A*, Pistillate flowers; *B*, staminate flowers.

PLATE XIV. Flowers from first-generation seedlings of crosses of pansies (natural size). *Viola tricolor* ♀ × cultivated forms ♂. The row of flowers at the bottom is *V. tricolor* uncultivated.

PLATE XV. Single branch of hybrid dahlia plant. *Dahlia chisolmii* ♀ × Twentieth Century dahlia ♂. Height of branch 7 feet 6 inches, showing very large compound leaves near the base and long flowering stems.

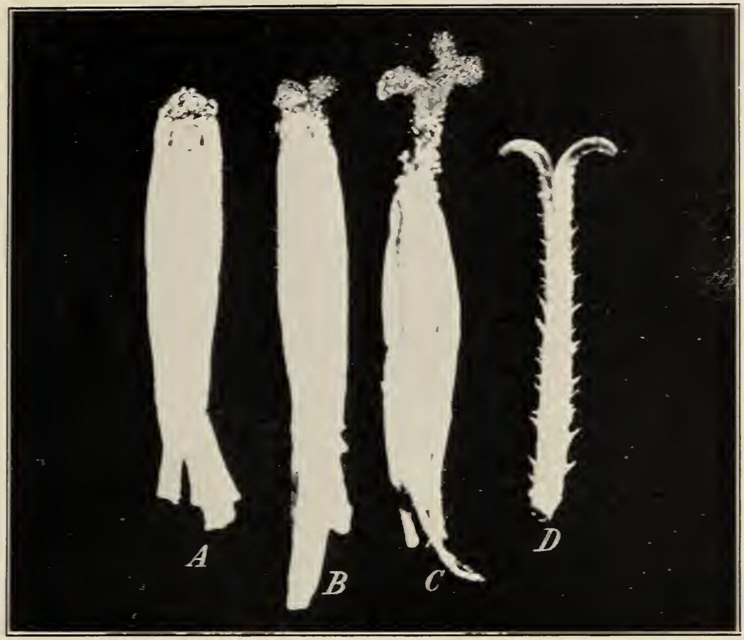

FIG. 1.—STAMINAL TUBES AND STIGMAS OF LETTUCE FLOWERS (ENLARGED TWENTY DIAMETERS).

FIG. 2.—DEPOLLINATING LETTUCE FLOWERS WITH WATER FROM A GARDEN HOSE.

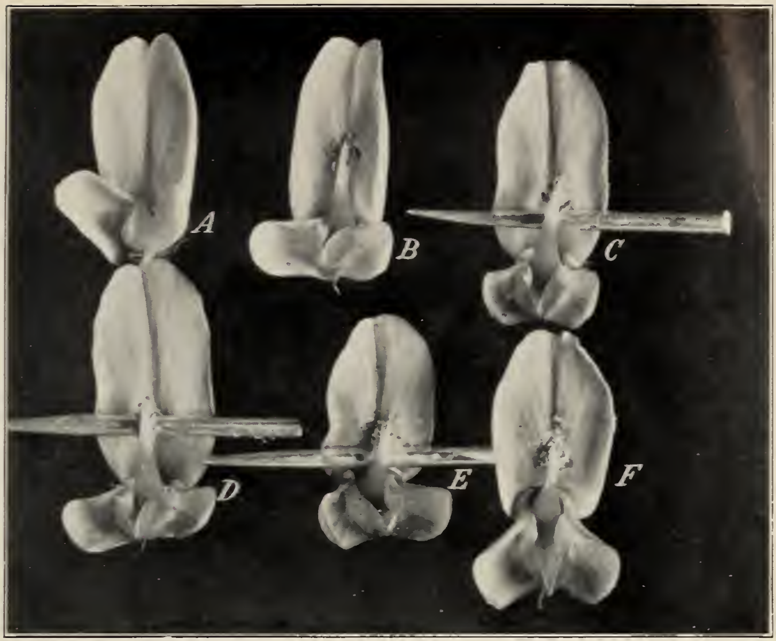

FIG. 1.—FLOWERS OF ALFALFA (ENLARGED FIVE DIAMETERS), SHOWING THE METHOD OF DEPOLLINATING AND CROSSING USED.

FIG. 2.—RACEME OF ALFALFA FLOWERS (ENLARGED SIX DIAMETERS).

SEXUAL COLUMNS OF ALFALFA FLOWERS (ENLARGED TEN DIAMETERS), SHOWING DIFFERENT STAGES OF DEVELOPMENT.

A and *B*, Columns with anthers just before the dehiscing stage; *C* and *D*, columns with anthers dehisced; *E* and *F*, columns with pollen and empty anthers removed by the aid of water previous to artificial pollination.

FIG. 1.—FLOWERS AND YOUNG PODS OF THE COWPEA (TWICE NATURAL SIZE).

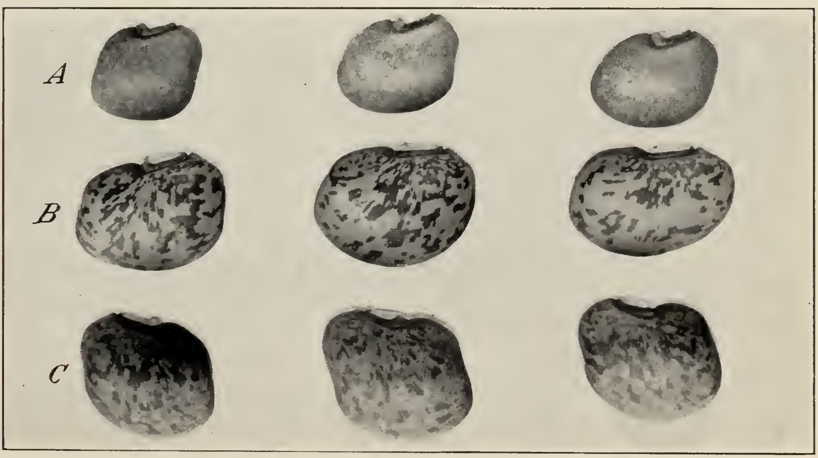

FIG. 2.—SEEDS OF COWPEA PARENTS AND OF A FIRST-GENERATION CROSS (ENLARGED TWO AND ONE-HALF DIAMETERS).

A, New Era; *B*, Whippoorwill; *C*, New Era ♀ × Whippoorwill ♂.

FIG. 1.—DAHLIA CHISOLMII (ENLARGED FOUR DIAMETERS), SHOWING DISK FLORETS BEFORE DEPOLLINATION.

FIG. 2.—DAHLIA CHISOLMII (ENLARGED FOUR DIAMETERS), SHOWING DISK FLORETS AFTER DEPOLLINATION BY A JET OF WATER.

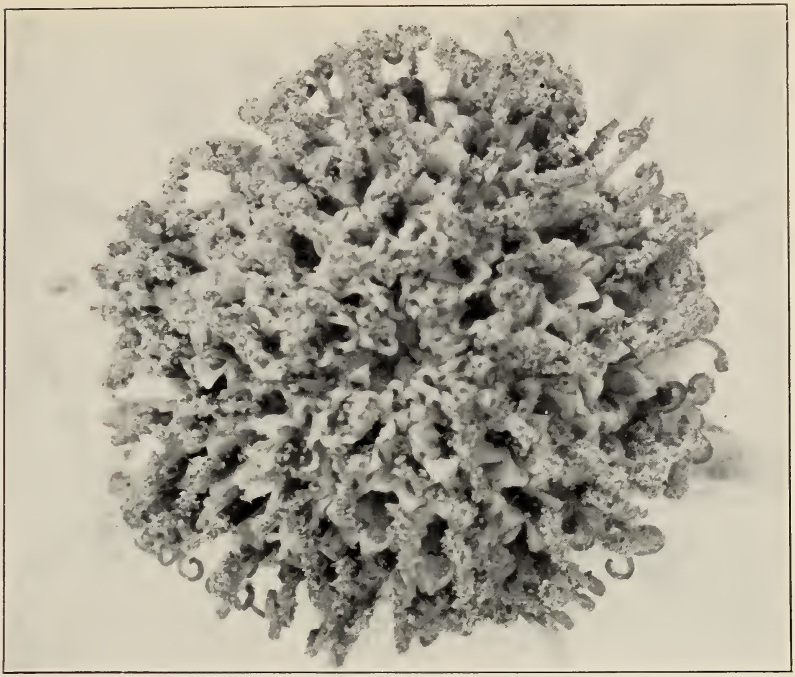

FIG. 1.—DISK FLORETS OF THE GREENHOUSE CINERARIA BEFORE DEPOLLINATION (ENLARGED FIVE DIAMETERS).

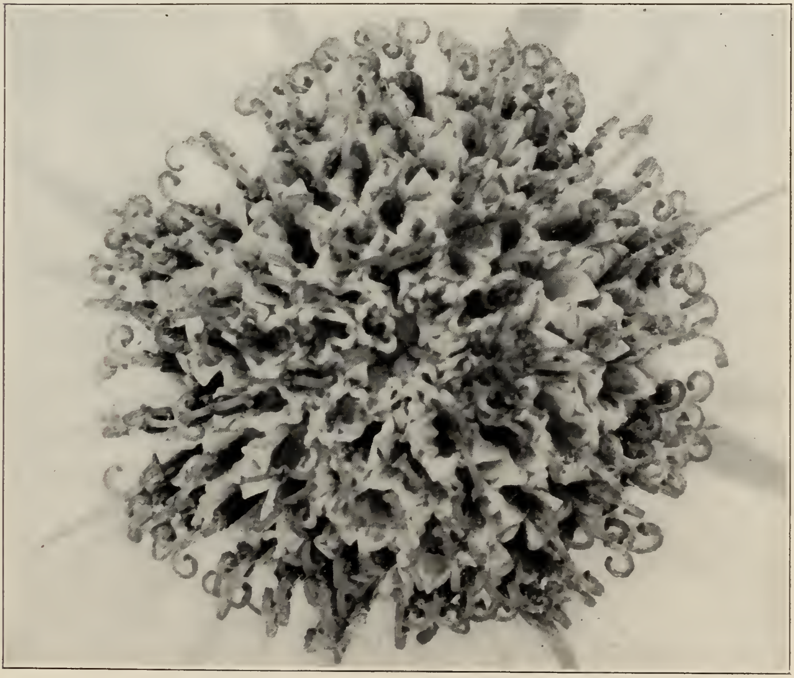

FIG. 2.—DISK FLORETS OF THE GREENHOUSE CINERARIA AFTER DEPOLLINATION (ENLARGED FIVE DIAMETERS).

FIG. 1.—FLOWER OF DAHLIA (ENLARGED FIVE DIAMETERS), SHOWING THE DISK FLORETS, THE STIGMAS OF WHICH ARE NEARLY ALL COVERED WITH POLLEN.

FIG. 2.—THE DAHLIA FLOWER (ENLARGED FIVE DIAMETERS) SHOWN IN FIGURE 1, WITH THE OUTER FLORETS DEPOLLINATED BY A JET OF WATER.

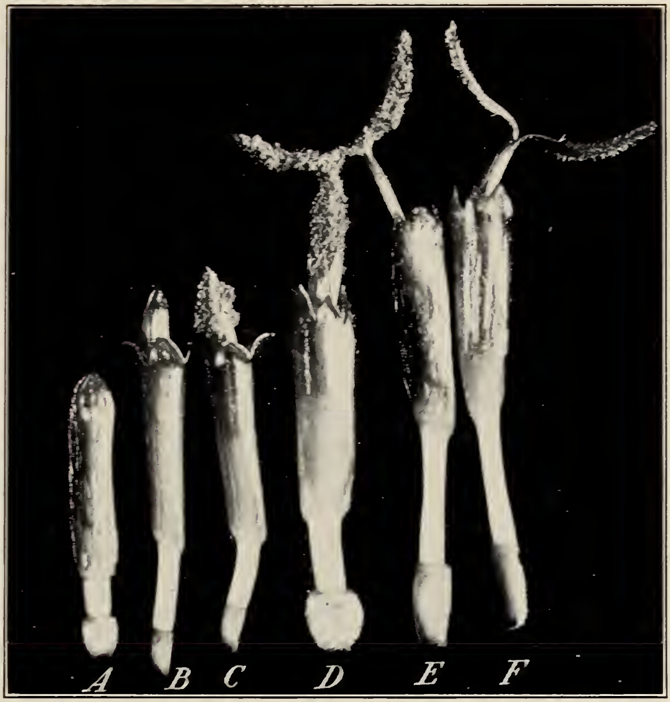

FIG. 1.—DAHLIA FLORETS (ENLARGED TEN DIAMETERS), SHOWING DEVELOPMENT OF THE STIGMAS.

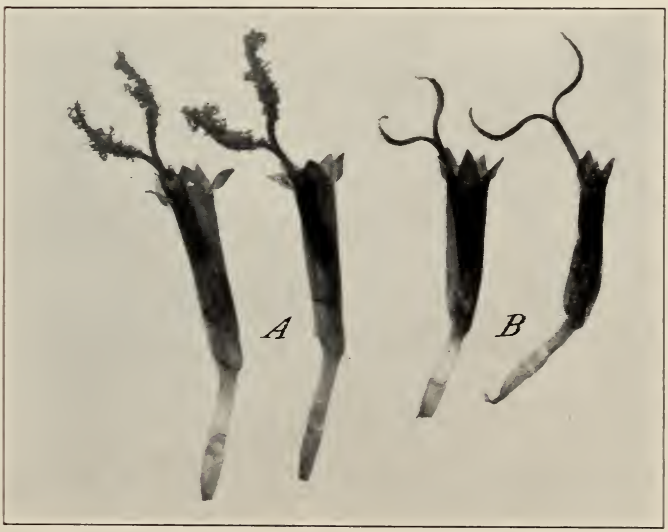

FIG. 2.—DAHLIA FLORETS (ENLARGED SIX DIAMETERS), BEFORE AND AFTER DEPOLLINATION.

FIG. 1.—DISK FLORETS OF HELIANTHUS (ENLARGED FIVE DIAMETERS), SHOWING DIFFERENT STAGES OF DEVELOPMENT.

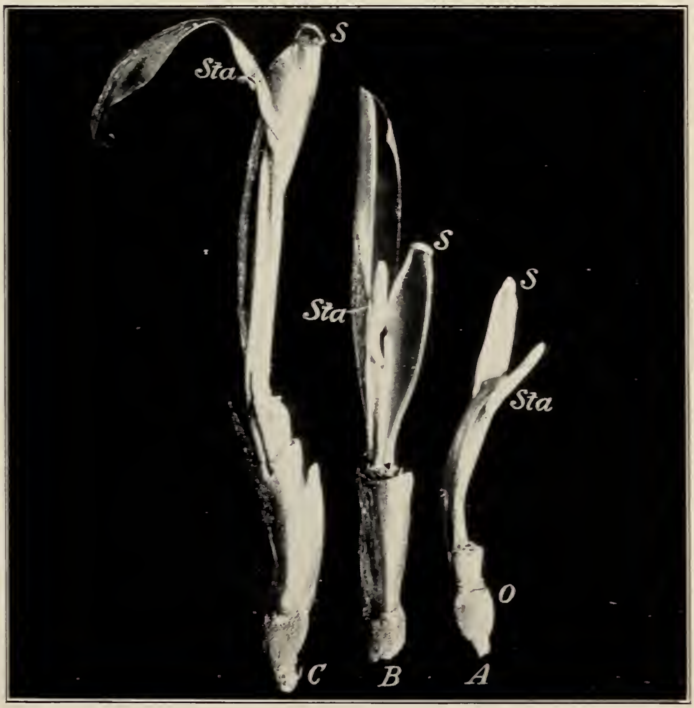

FIG. 2.—FLOWERS OF CANNA INDICA (NATURAL SIZE), SHOWING STAMEN AND PISTIL IN DIFFERENT STAGES OF DEVELOPMENT.

FIG. 1.—FLOWERS (PROTEROGYNOUS) OF PLANTAGO LANCEOLATA, SHOWING PISTILS MATURING BEFORE THE STAMENS.

FIG. 2.—FLOWER (PROTERANDROUS) OF CAMPANULA ROTUNDIFOLIA (ENLARGED THREE DIAMETERS), SHOWING THE STAMENS MATURING BEFORE THE PISTIL.

FIG. 1.—SINGLE TEA ROSE, SHOWING HOW EASILY HERMAPHRODITE FLOWERS HAVING LARGE STAMENS MAY BE EMASCULATED.

FIG. 2.—FLOWERS OF ROSES (NATURAL SIZE), SHOWING METHOD OF EMASCULATION.

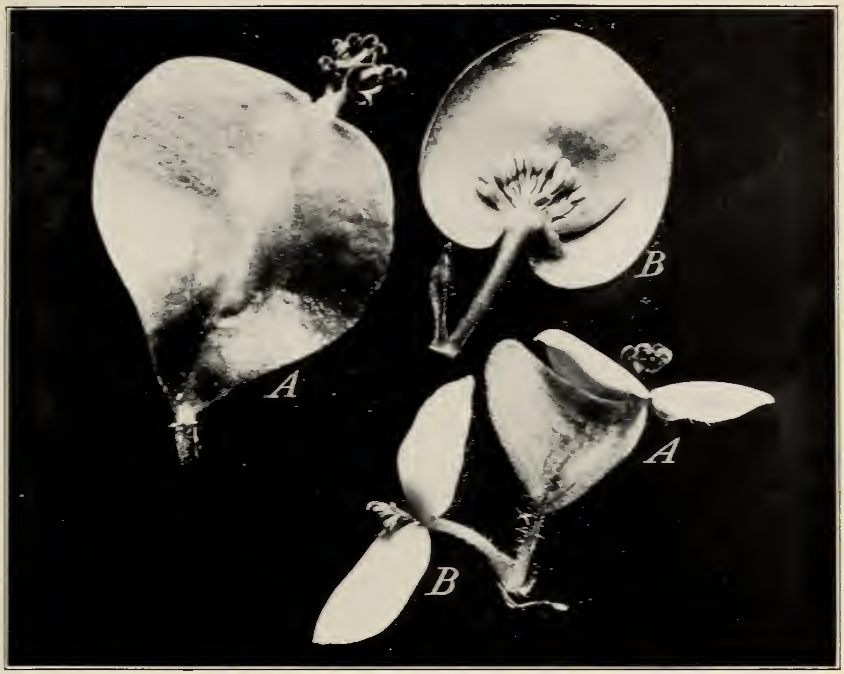

FIG. 1.—FLOWERS (MONŒCIOUS) OF THE BEGONIA, SHOWING THE SEXES IN DIFFERENT FLOWERS ON THE SAME PLANT.

FIG. 2.—STAMINATE AND PISTILLATE FLOWERS OF CODIAEUM VARIEGATUM FROM THE SAME PLANT.

FIG. 1.—STAMINATE AND PISTILLATE (DIŒCIOUS) FLOWERS OF WILLOW, FROM DIFFERENT PLANTS.

FIG. 2.—FLOWERS FROM STAMINATE AND PISTILLATE PLANTS OF ASPARAGUS OFFICINALIS (ENLARGED FIVE DIAMETERS).

FLOWERS FROM FIRST-GENERATION SEEDLINGS OF CROSSES OF PANSIES (NATURAL SIZE).

Viola tricolor ♀ × cultivated forms ♂. The row of flowers at the bottom is *Viola tricolor* uncultivated.

SINGLE BRANCH OF HYBRID DAHLIA PLANT.

Dahlia chisolmii ♀ × Twentieth Century dahlia ♂.

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